AmoyDx® Virus/Cell RNA Kit

(Spin Column)

For virus and cells RNA purification

Instruction for Use

For Research Use Only

REF 8.0250301X036G 36 tests

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Intended Use

The AmoyDx® Virus/Cell RNA Kit provides silica-based membrane and special lysis buffer system for virus and cell RNA extraction effectively. This kit is specially designed for isolation and purification of RNA from biological specimens and cultured cells. The purified RNA is suitable for downstream applications such as real-time quantitative PCR (qPCR).

Principles of the Procedure

The biological specimens and cultured cells are lysed with Buffer RLB and Proteinase K Solution Plus to release RNA. The lysate is applied to a RNA spin column, where the RNA binds to the membrane and impurities are removed with wash buffer. The purified RNA is eluted in Buffer RTE.

Kit Contents

This kit contains sufficient reagents to perform 36 tests (Table 1).

Table 1 Kit Contents

Tube No.	Component	Quantity
—	**RNA Spin Columns**	36 tubes/bag ×1
—	**Collection Tubes (2 mL)**	72 tubes/bag ×1
—	**Centrifugal Tubes (1.5 mL)**	72 tubes/bag ×1
1	**Buffer RLB**	31 mL/vial ×1
2	**Proteinase K Solution Plus**	850 μL/tube ×1
3	**Wash Buffer A (concentrate)**	13 mL/vial ×1
4	**Wash Buffer B (concentrate)**	6 mL/vial ×1
5	**Buffer RTE**	1.5 mL/tube ×2

Note:

1) ***Buffer RLB*** *contains guanidine salt, not compatible with disinfectants containing bleach or acidic solutions.*
2) *For the first time use, add 17 mL and 24 mL of absolute ethanol respectively into* ***Wash Buffer A (concentrate)*** *and* ***Wash Buffer B (concentrate)****, mix each of them thoroughly. Tick the check box on the bottle label.*

Storage and Stability

The shelf life of the kit is 12 months. The kit should be stored dry at room temperature (10~30℃).

Additional Reagents and Equipment Required but Not Suppliedr

1) Ethanol (96~100%).
2) Heating block (80℃ adjustable) .
3) Microcentrifuge (1.5 mL rotor and 12,000×g adjustable).
4) Vortexer.
5) Palm centrifuge.
6) Nuclease-free centrifugal tubes.
7) Adjustable pipettors and sterile, nuclease-free pipet tips.

Precautions and Handling Requirements

Precautions

- Please read the instruction carefully and become familiar with all components of the kit prior to use, and strictly follow the instruction during operation.
- DO NOT use the kit or any kit component after their expiry date.
- DO NOT use any other reagents from different lots in the tests.
- DO NOT use any other reagent in the other test kits.

Safety Information

- ***Buffer RLB*** contain guanidine salt, which can form highly reactive compounds when combined with bleach. **Do not add bleach or acidic solutions directly to the sample-preparation waste.** If the liquid containing this buffer is spilt, clean with suitable laboratory detergent and water.
- Handle all specimens and components of the kit as potentially infectious material using safe laboratory procedures.
- Only trained professionals can use this kit. Please wear suitable lab coat and disposable gloves while handling the reagents.
- Perform all the procedures in the biosafety cabinet.
- If a spill contains potentially infectious reagents, clean the affected area first with laboratory detergent and water, then with 1% (v/v) sodium hypochlorite or a suitable laboratory disinfectant.
- Avoid skin, eyes and mucous membranes contact with the chemicals. In case of contact, flush with water immediately.
- DO NOT pipet by mouth.

Decontamination and Disposal

- Gloves should be worn and changed frequently when handling samples and reagents to prevent contamination.
- Using separate, dedicated pipettes, nuclease-free centrifugal tubes and nuclease-free filtered pipette tips when handling samples and reagents to prevent the RNase contamination and cross-contamination.
- All disposable materials are for one time use. DO NOT reuse.
- The unused reagents, used kit, and waste must be disposed of properly.

Cleaning

- After the experiment, wipe down the work area, spray down the pipettes and equipment with 75% ethanol or 10% hypochlorous acid solution.

Starting Material

1. Biological specimens include respiratory specimen, saliva, swabs, and samples stored in storage reagent.
2. The samples should be extracted RNA immediately. For short-term storage of up to 24 hours, the sample should be stored at 4℃. For long-term storage of over 24 hours, the sample should be stored at or below -20℃ and avoid repeated freezing-thawing.
3. The infectious specimens should be transported with separate container and stored at separate freezer.

Assay Procedure

1. Sample Pretreatment

1) For respiratory specimen and saliva: mix the samples by vortexing, pipet 300 μL sample into a clean 1.5 mL centrifugal tube, then add 600 μL **Buffer RLB**.
   Note: *cut a small tail (0.5~1 cm) of the pipet tip if the specimen is too viscous to pipet.*
2) For swabs not stored in preservation reagent: cut the swab tip into a clean 1.5 mL centrifugal tube, add 800 μL **Buffer RLB** to allow the lysate fully immerse the swab tip (Do not discard the swab tip, continue with the RNA extraction steps).
3) For specimen collected in preservation reagent:
   a) If sample stored in AmoyDx preservation reagent: mix the sample by vortexing, pipet 900 μL sample into a clean 1.5 mL centrifugal tube.
   b) If sample stored in other preservation reagent: mix the sample by vortexing, pipet 300 μL sample into a clean 1.5 mL centrifugal tube, and then add 600 μL **Buffer RLB**.

2. RNA Extraction

Note:

- *For the first time use, add 17 mL and 24 mL of absolute ethanol respectively into* ***Wash Buffer A (concentrate)*** *and* ***Wash Buffer B (concentrate)****, mix each of them thoroughly. Mark them clearly.*
- *Before the RNA extraction, please check the reagents without leakage. Shake the reagents gently to mix the solutions. If the reagents contain precipitates, dissolved by heating at 50 ℃.*

1) Add 20 μL **Proteinase K Solution Plus** into the sample tube, close the lid and mix the solutions by vortexing for 15 seconds, then briefly centrifuge for 5~10 seconds. Transfer the sample tube into heating block and incubate at 56℃ for 10 min.
   Note: *if the heating block has the shaking function, please incubate the tube at 500 rpm.*
2) Adjust the temperature of heating block as 80℃, incubate the tube at 80℃ for 10 min, then take out the tube at room temperature.
   Note: *for centrifuge tube with the swab tip, transfer all the above solutions of sample tube into a clean 1.5 mL centrifugal tube.*
3) Add 600 μL **Ethanol** (96~100%) into the lysate, mix the solutions by inverting the tube 10 times, then briefly centrifuge for 5~10 seconds.
   Note: *if there is obvious precipitate in the tube, centrifuge the tube at 8,000×g for 1 min and pipet the supernatant into the RNA Spin Column.*
4) Transfer 750 μL supernatant into the RNA Spin Column (in a 2 mL collection tube) without wetting the rim, close the lid, and centrifuge at 12,000×g for 30 seconds. Discard the flow-through in collection tube.
5) Transfer the remaining supernatant into the RNA Spin Column without wetting the rim, close the lid, and centrifuge at 12,000×g for 30 seconds. Discard the flow-through in collection tube.
6) Add 550 μL **Wash Buffer A** into RNA Spin Column, centrifuge at 12,000×g for 30 seconds. Discard the flow-through in collection tube.
7) Add 550 μL **Wash Buffer B** into RNA Spin Column, centrifuge at 12,000×g for 30 seconds. Discard the collection tube with flow-through.
8) Place the RNA Spin Column in a clean 2 mL collection tube. Centrifuge at 12,000×g for 2 min, and discard the collection tube.
9) Place the RNA Spin Column in a clean 1.5 mL centrifugal tube. Keep the RNA Spin Column open and incubate at 56℃ for 2 min.
10) Apply 40 μL **Buffer RTE** to the center of the membrane. Do not touch the membrane. Close the lid and incubate at 56℃ for 2 min. Centrifuge at 12,000×g for 1 min.
11) The eluted RNA in the centrifugal tube is ready for use immediately. Store the RNA at or below -20℃ if it's not used within 2 hours.
    Note: *Buffer RTE is only for elution and storage of RNA, NOT for other use.*

Limitations

1) The quality of extracted RNA is subject to the influence of such factors as sample source, sampling process, collection site, storage conditions.
2) Sample quality has a high impact on quality and amount of the purified RNA.
3) The presence of RNase in the laboratory environment may degrade RNA during or after the purification procedure. All the equipment, consumables and workbench should be treated before use to ensure that it is RNase-free.

Symbols

	Manufacturer		Catalogue Number
	Batch Code		Use By
	Contains Sufficient for <n> Tests		Temperature Limitation
	Consult Instructions For Use		Keep Dry
	This Way Up		Fragile, Handle With Care